NC STATE UNIVERSITY

Science-Policy Interface session:

Epidemiological modeling in livestock

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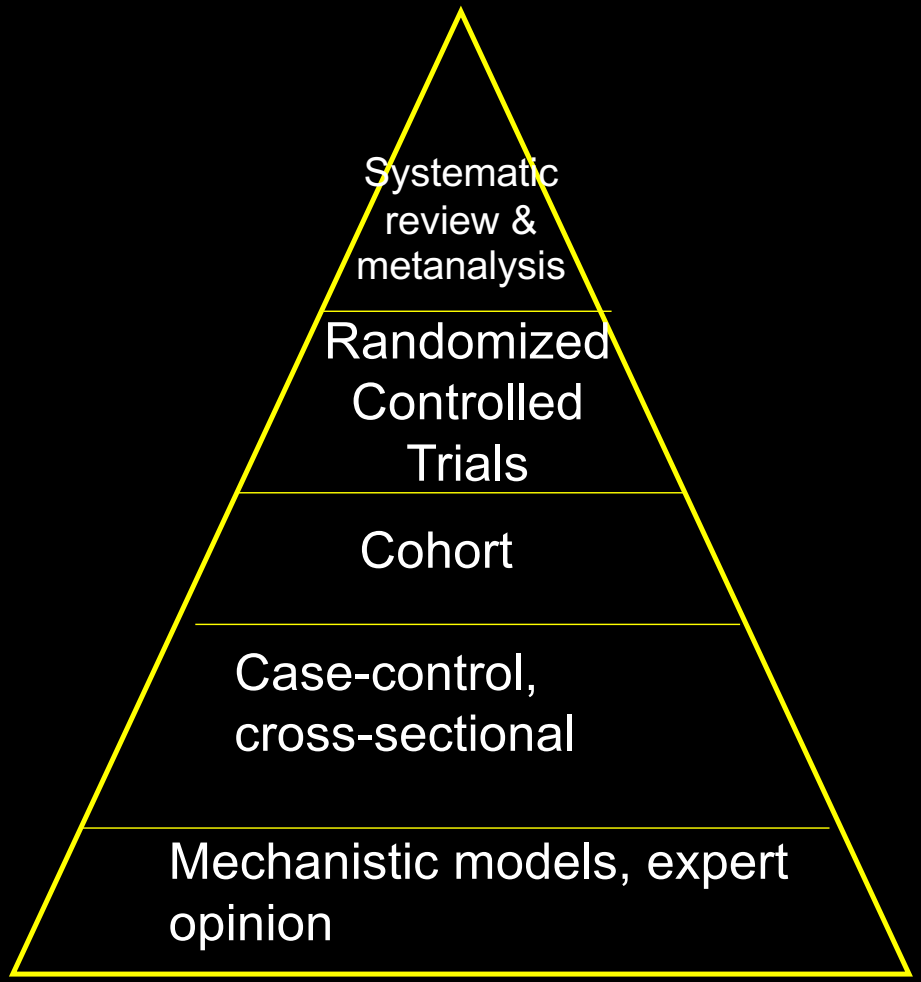
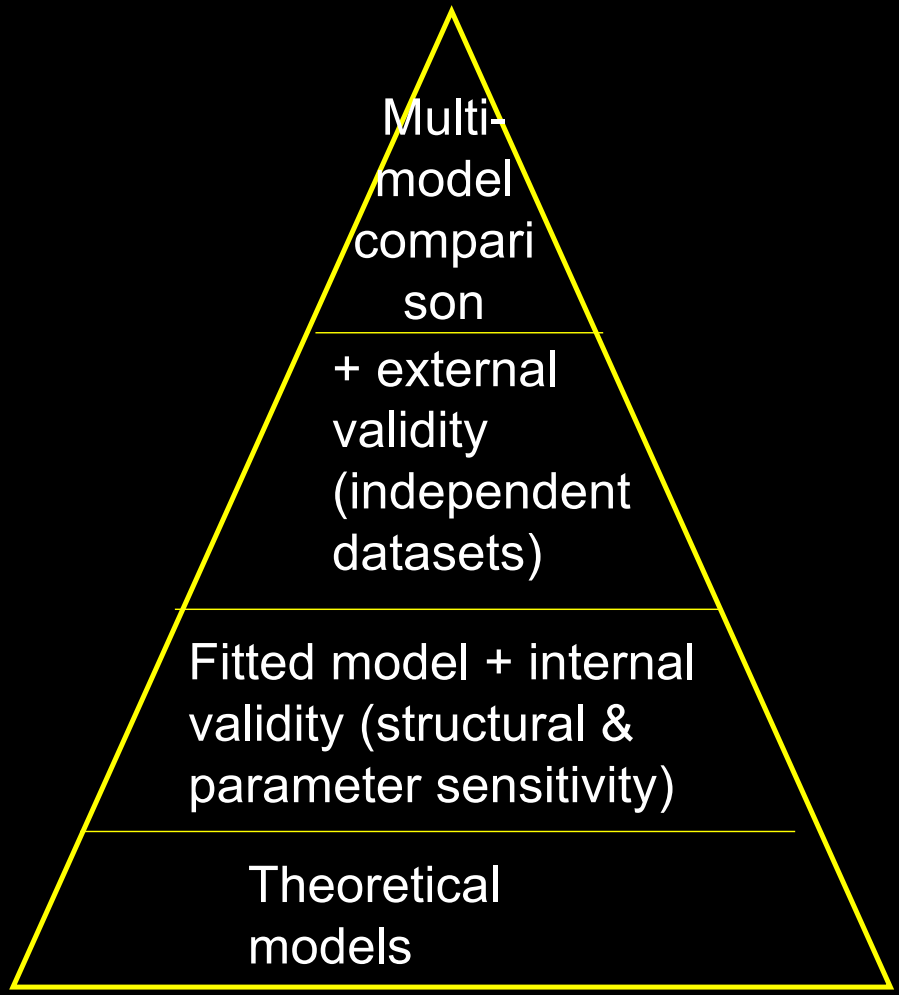
Mathematical modeling for policy

- Explicit integration of scientific evidence on the many factors relevant to a decision.
- Evaluation of interventions (counterfactuals, what if)
- Estimate key parameters and outcomes that often are unobserved (transmission & fitness).
- By identifying the assumptions and uncertainties to which decision making is most sensitive, models can help to prioritize further data collection and research.

How do we measure evidence from models?



Epidemiological evidence



Modeling evidence

US National Action Plan for Combating Antibiotic-Resistant Bacteria, 2020-2025

Goal 1: Slow the Emergence of Resistant Bacteria and Prevent the Spread of Resistant Infections

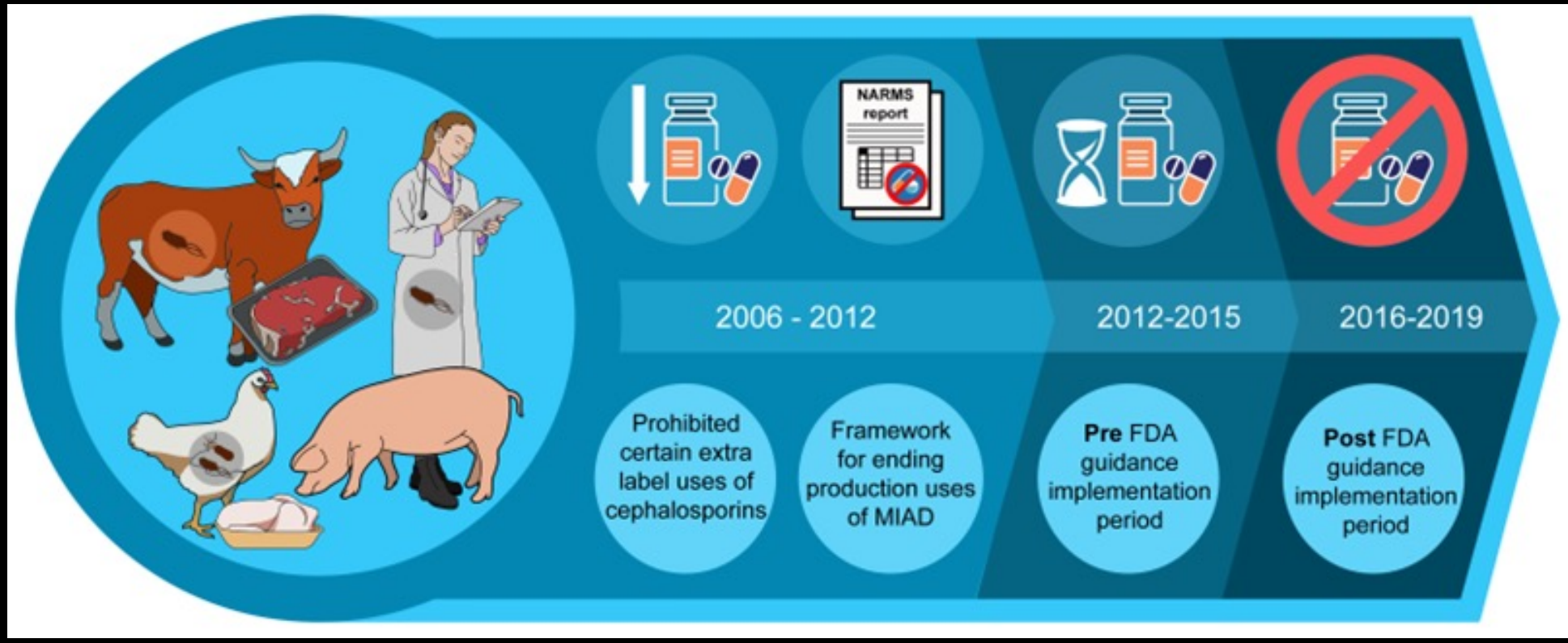
Goal 2: Strengthen National One Health Surveillance Efforts to Combat Resistance

Goal 3: Advance Development and Use of Rapid and Innovative Diagnostic Tests for Identification and Characterization of Resistant Bacteria

Goal 4: Accelerate Basic and Applied Research and Development for New Antibiotics, Other Therapeutics, and Vaccines

Goal 5: Improve International Collaboration and Capacities for Antibiotic-resistance Prevention, Surveillance, Control and Antibiotic Research and Development.

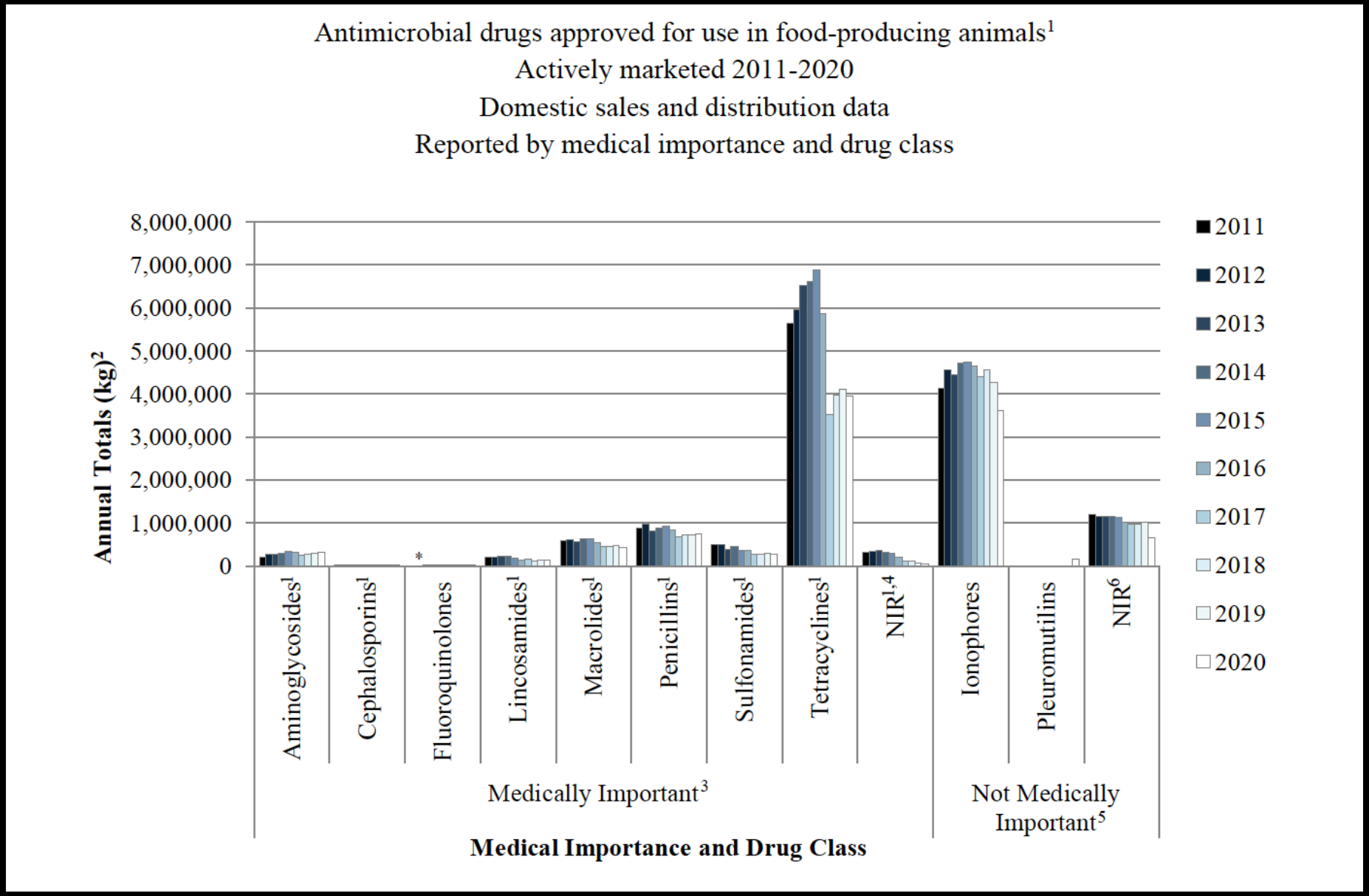
Timeline of FDA policies and guidance on antimicrobial resistance for food animals



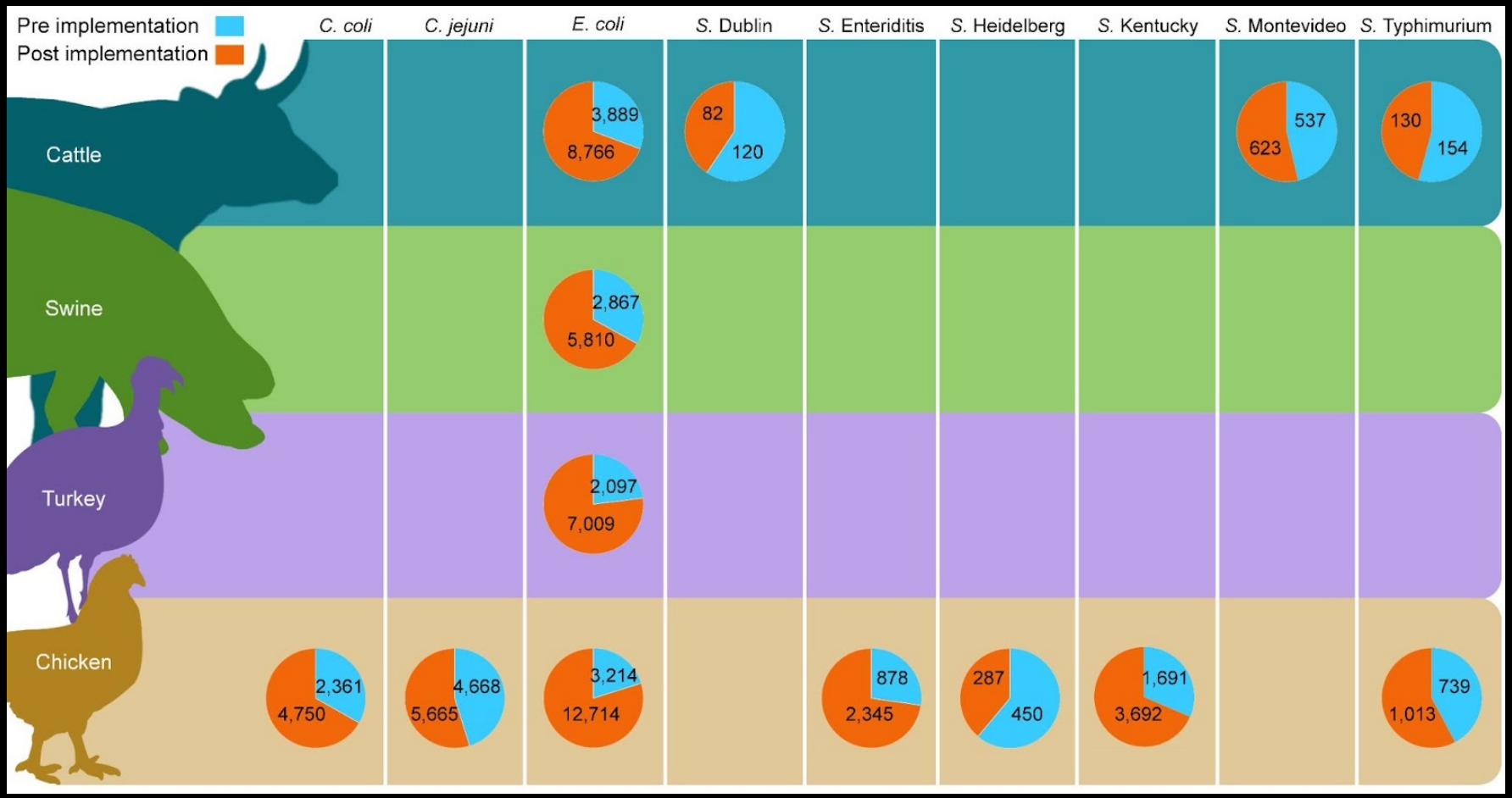
*VFD= Veterinary feed directive, MIA= Medically important antimicrobials. MIAD= Medically important animal drugs

Figure by Manuel Jara, 2022

Antimicrobial use in food animals- FDA sales data



Number and proportion of isolates from the National Antimicrobial Monitoring System collected from the pre-implementation (2012-2015) and post-implementation period (2016-2019) sorted by animal and bacterial host.

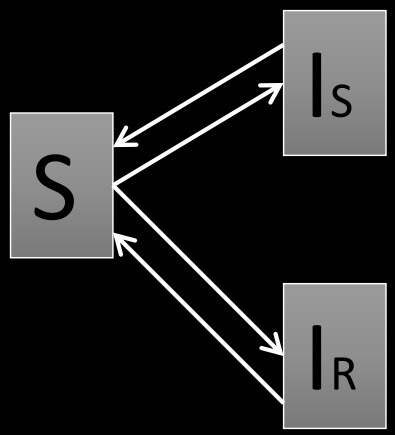


Chandra Deb et al., in review

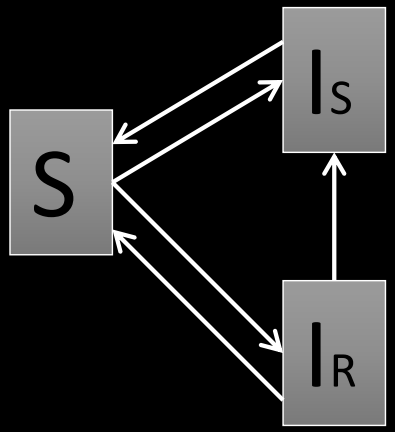
- For all animal-bacteria groups, significant decreases in MIC during post-implementation were less than 1 fold minimum inhibitory concentration dilution.
- Changes not consistent across outcomes (% resistance, MIC, resistant genes)
- What effect size? How long?
- Isolate a single intervention from other effects is difficult

Long-term effects of antimicrobial stewardship interventions: Antimicrobial resistance reversion?

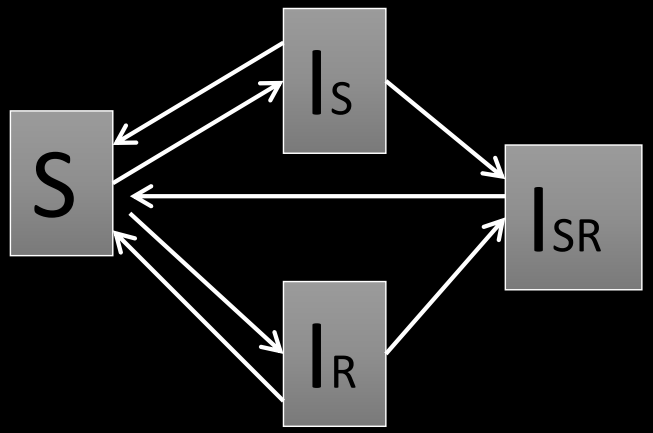
- Fitness cost of resistance
- Compensatory adaptation
- Co-selection (other antimicrobials, biocides, heavy metals…)
- Presence of susceptible genotypes



Competitive exclusion



Superinfection



Coinfection

Population-level fitness

$R_{0\ \text{resistant}} < R_{0\ \text{sensitive}}$ (↓transmissibility, ↓duration of infection)

Relative fitness often unknown

Current Landscape of AMR Research

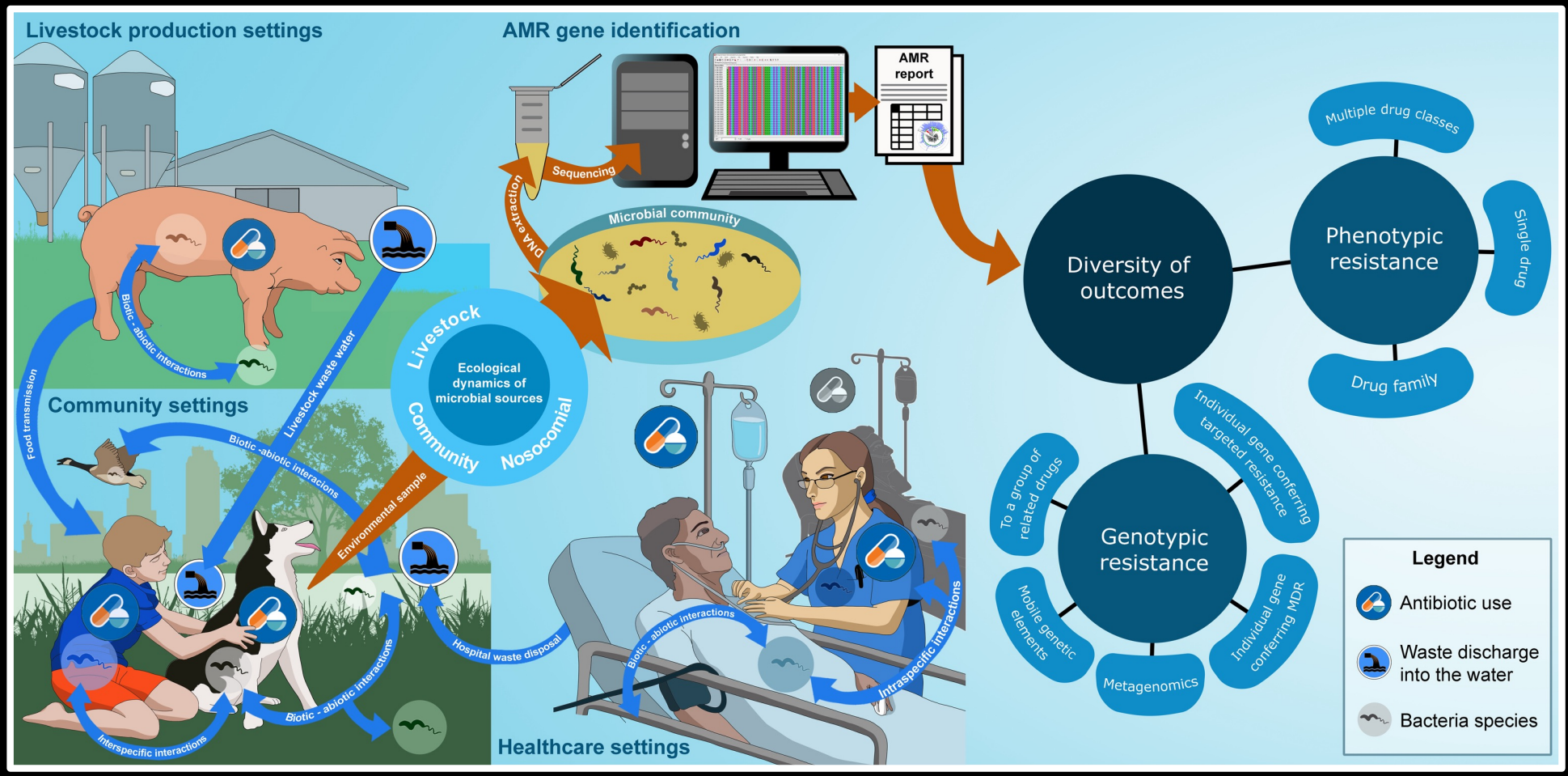
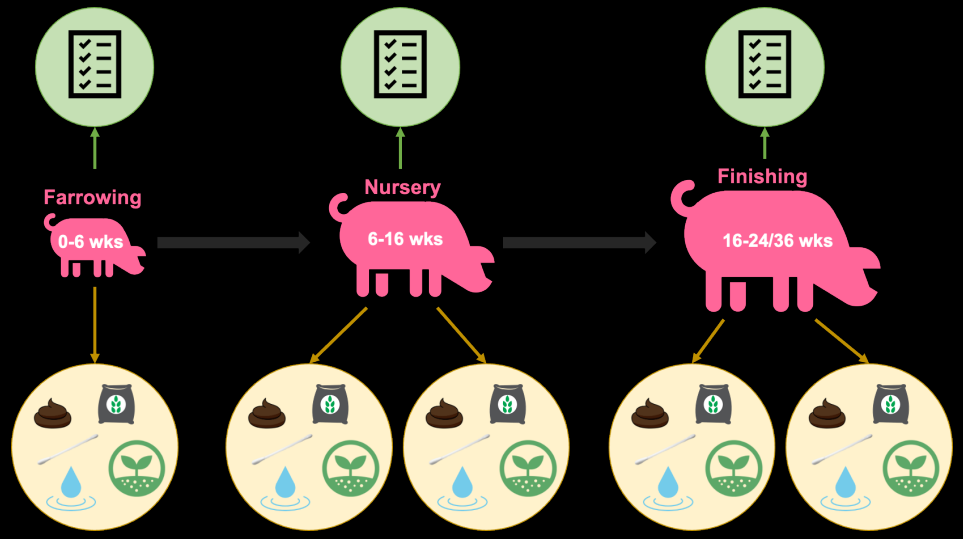


Figure by Manuel Jara (2022)

Campylobacter coli from Swine Farms

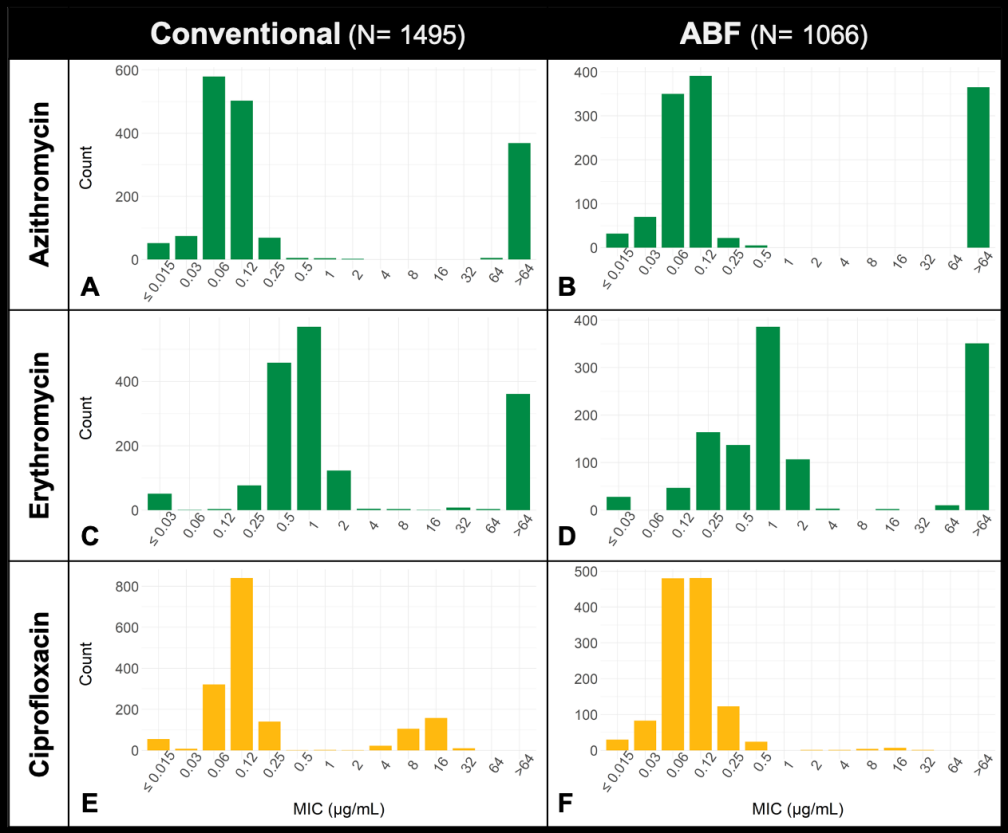
- Prior research on AMR in conventional & antibiotic-free (ABF) production systems
- Samples collected from NC pig cohorts & their environment from birth till death
- Samples cultured for *Campylobacter* spp.
  - 2900 *C. coli* isolates phenotypically characterized
  - 1300 C. coli isolates sequenced using Illumina MiSeq

Conventional	Antibiotic Free (ABF)
10 cohorts	8 cohorts
Antibiotics given	Antibiotics never given
Reared indoors	Reared outdoors
Slaughtered ~6 months	Slaughtered ~9 months



Fitness Effects of AMR in Experimental vs Natural *C. coli* Populations

- Our phenotype data do not support broadly accepted fitness effects
  - ***23S rRNA* A2075G mutation**
  - ***gyrA* T86I mutation**
- Experimental studies have highly controlled exposures, in general
- Natural bacteria exposed to diverse, uncontrolled factors that can influence relative fitness effects of a genotype



Phylodynamic Approaches and Multi-Type Birth-Death Models

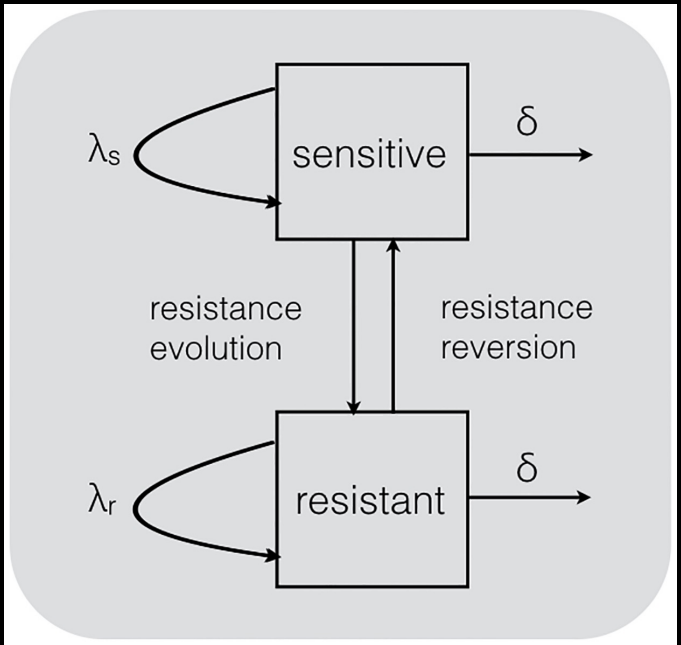
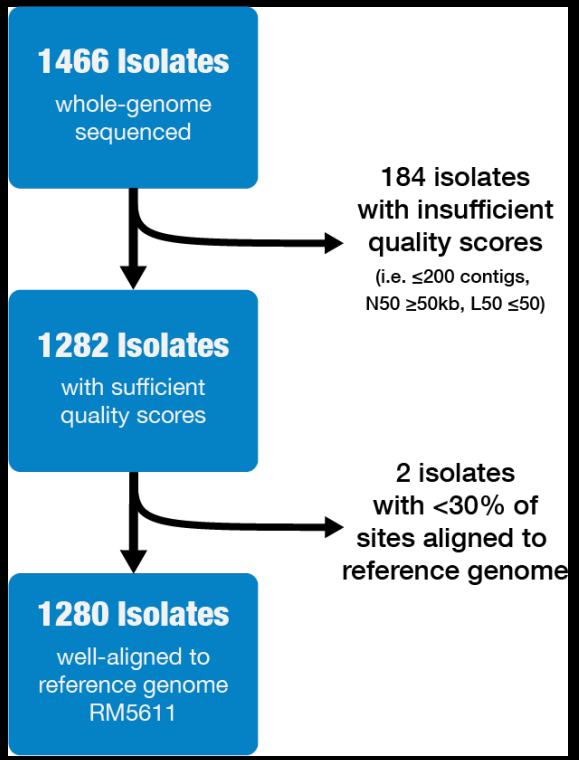


Figure from Kühnert et al. 2018

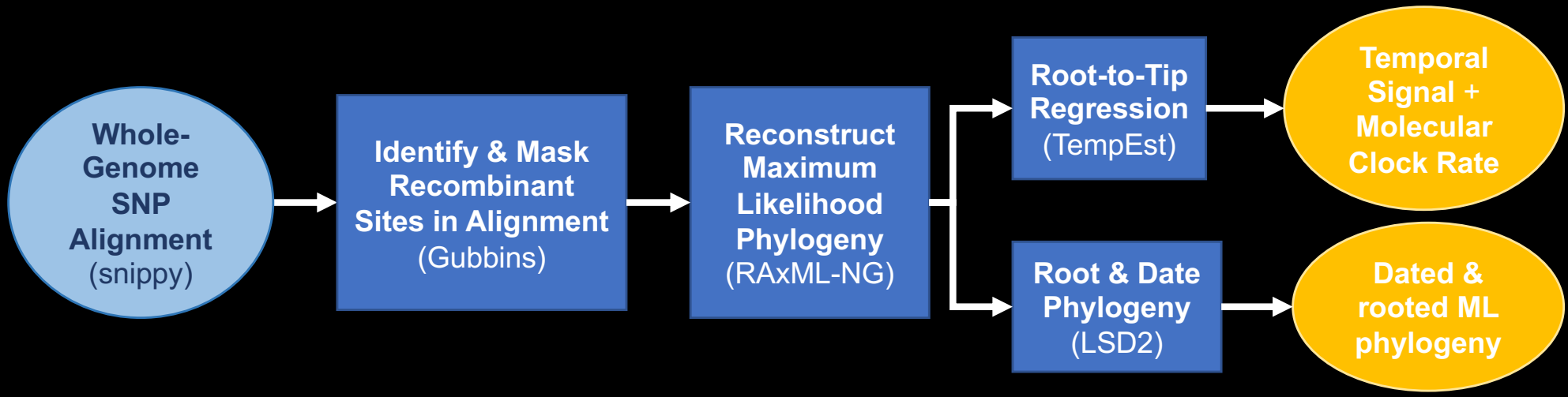
- **Phylodynamics** = integration of phylogenetic & epidemiological data to infer evolutionary processes (e.g. fitness)
- **Birth-death models** estimate birth rates of lineages based on branching rates in phylogeny
- **Multi-type birth death models** allow birth rate estimates of lineage to differ based on "type" (e.g. resistance or susceptibility)

Analytical Stage 1: Bioinformatic Analysis

1. Downloaded FASTQ files for all study isolates
2. *de novo* assembled genomes with Shovill implementation of SPAdes
3. Annotated genomes with Prokka
4. Screened for resistance genes using AMRFinderPlus
5. Ascertained sequence quality scores using Quast
6. Identified RM5611 as best reference in RefSeq based on Mash distances
7. Produced multiple sequence alignment against reference and cleaned alignment using Snippy

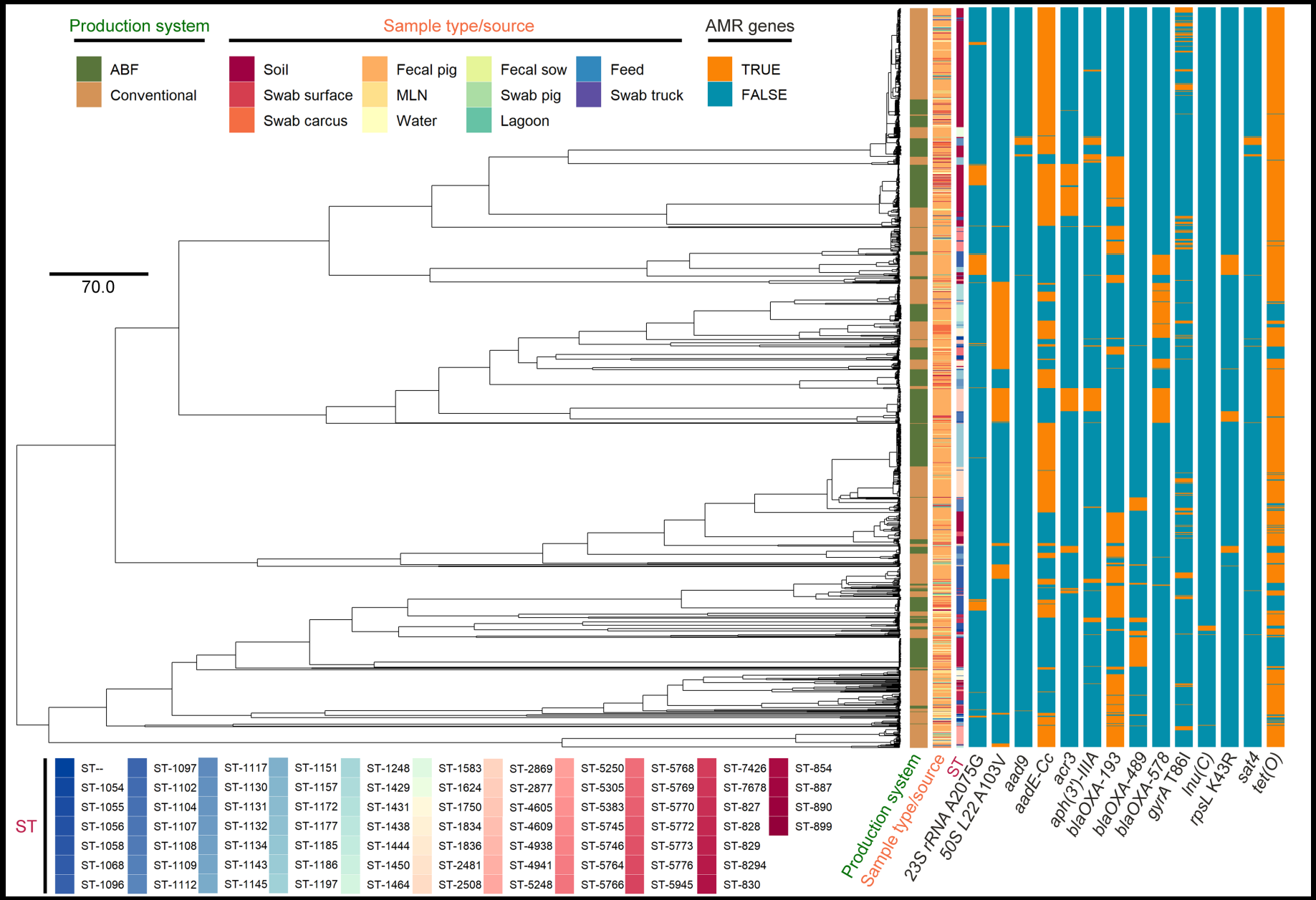


Analytical Stage 2: Phylogenetic Analysis



Analytical Stage 3: Phylodynamic Analysis

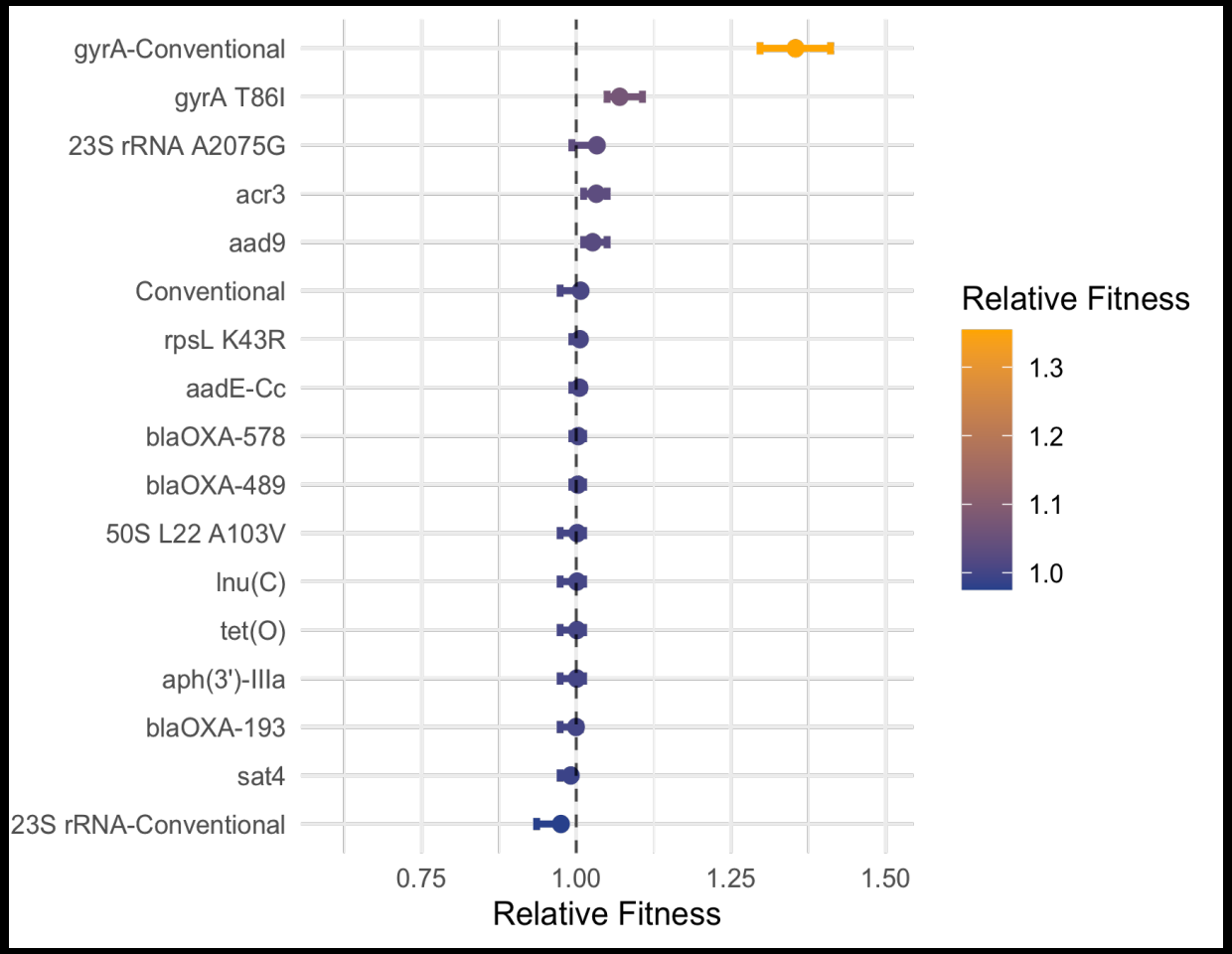
1. Used dated & rooted ML Phylogeny as input
2. Reconstructed ancestral states using PastML
3. Estimated birth rate using likelihood-based MTBD model framework
   - Consistent Death Rate = 1.267
   - Consistent Sampling Proportion = 0.1164
4. Inferred relative fitness for each feature & estimated 95% credible intervals
   - >1.0: advantageous fitness effect
   - =1.0: neutral fitness effect
   - <1.0: deleterious fitness effect


Production system
ABF
Conventional
Sample type/source
Soil
Swab surface
Swab carcus
Fecal pig
MLN
Water
Fecal sow
Swab pig
Lagoon
Feed
Swab truck
AMR genes
TRUE
FALSE
70.0
ST
ST-- ST-1054 ST-1055 ST-1056 ST-1058 ST-1068 ST-1096
ST-1097 ST-1102 ST-1104 ST-1107 ST-1108 ST-1109 ST-1112
ST-1117 ST-1130 ST-1131 ST-1132 ST-1134 ST-1143 ST-1145
ST-1151 ST-1157 ST-1172 ST-1177 ST-1185 ST-1186 ST-1197
ST-1248 ST-1429 ST-1431 ST-1438 ST-1444 ST-1450 ST-1464
ST-1583 ST-1624 ST-1750 ST-1834 ST-1836 ST-2481 ST-2508
ST-2869 ST-2877 ST-4605 ST-4609 ST-4938 ST-4941 ST-5248
ST-5250 ST-5305 ST-5383 ST-5745 ST-5746 ST-5764 ST-5766
ST-5768 ST-5769 ST-5770 ST-5772 ST-5773 ST-5776 ST-5945
ST-7426 ST-7678 ST-827 ST-828 ST-829 ST-8294 ST-830
ST-854 ST-887 ST-890 ST-899
Production system
Sample type/source
ST
23S rRNA A2075G
50S L22 A103V
aad9
aadE-Cc
acr3
aph(3')-IIIA
blaOXA-193
blaOXA-489
blaOXA-578
gyrA T86I
lnu(C)
rpsL K43R
sat4
tet(O)

Fitness Effects of *23S rRNA* & *gyrA* Mutations among *C. coli* from Conventional Farms

Model with all main effects plus two interaction terms:

- *gyrA* T86I X Conventional
- *23S rRNA* A2075G X Conventional

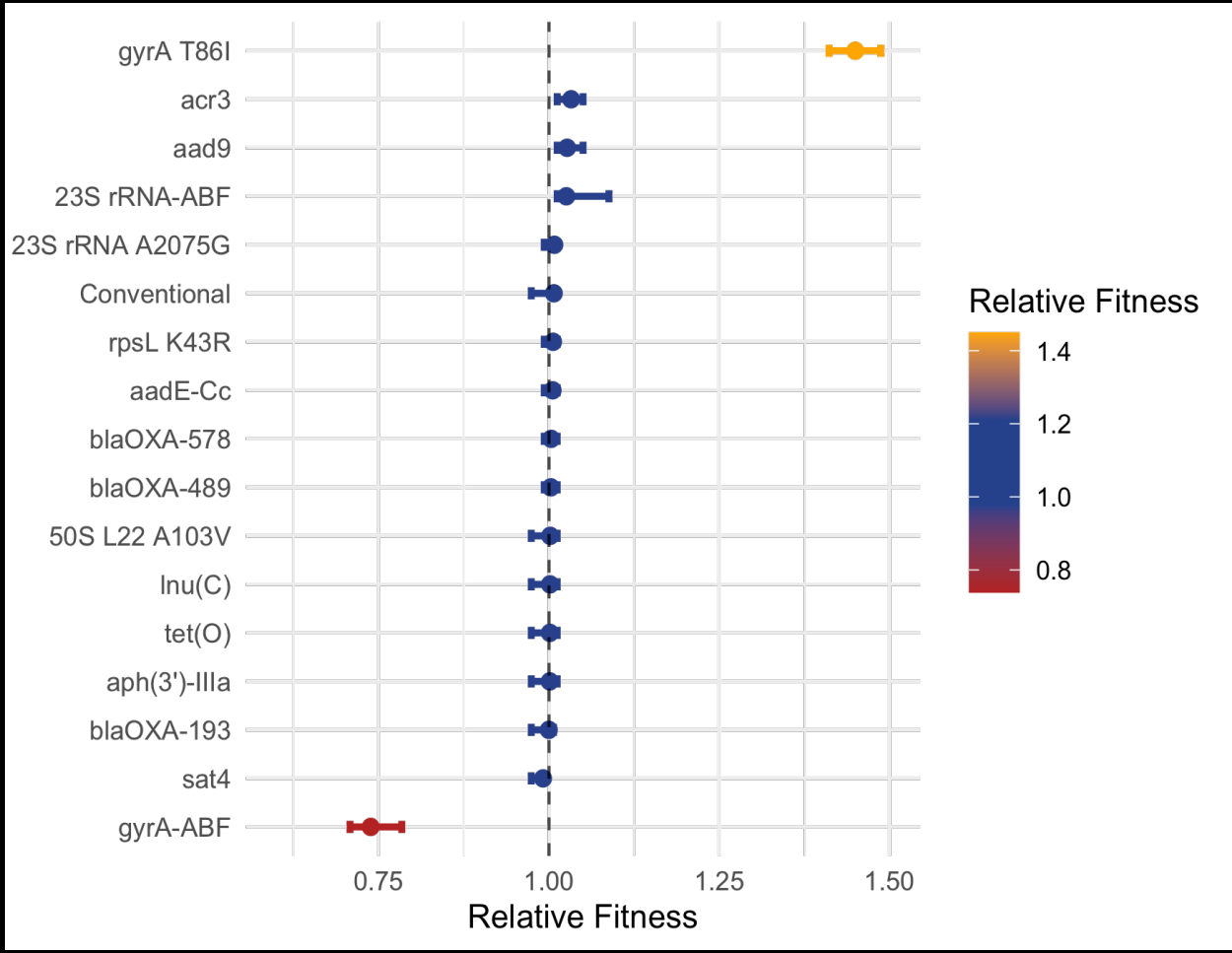


Fitness Effects of *23S rRNA* & *gyrA* Mutations among *C. coli* from ABF Farms

(alternative view)

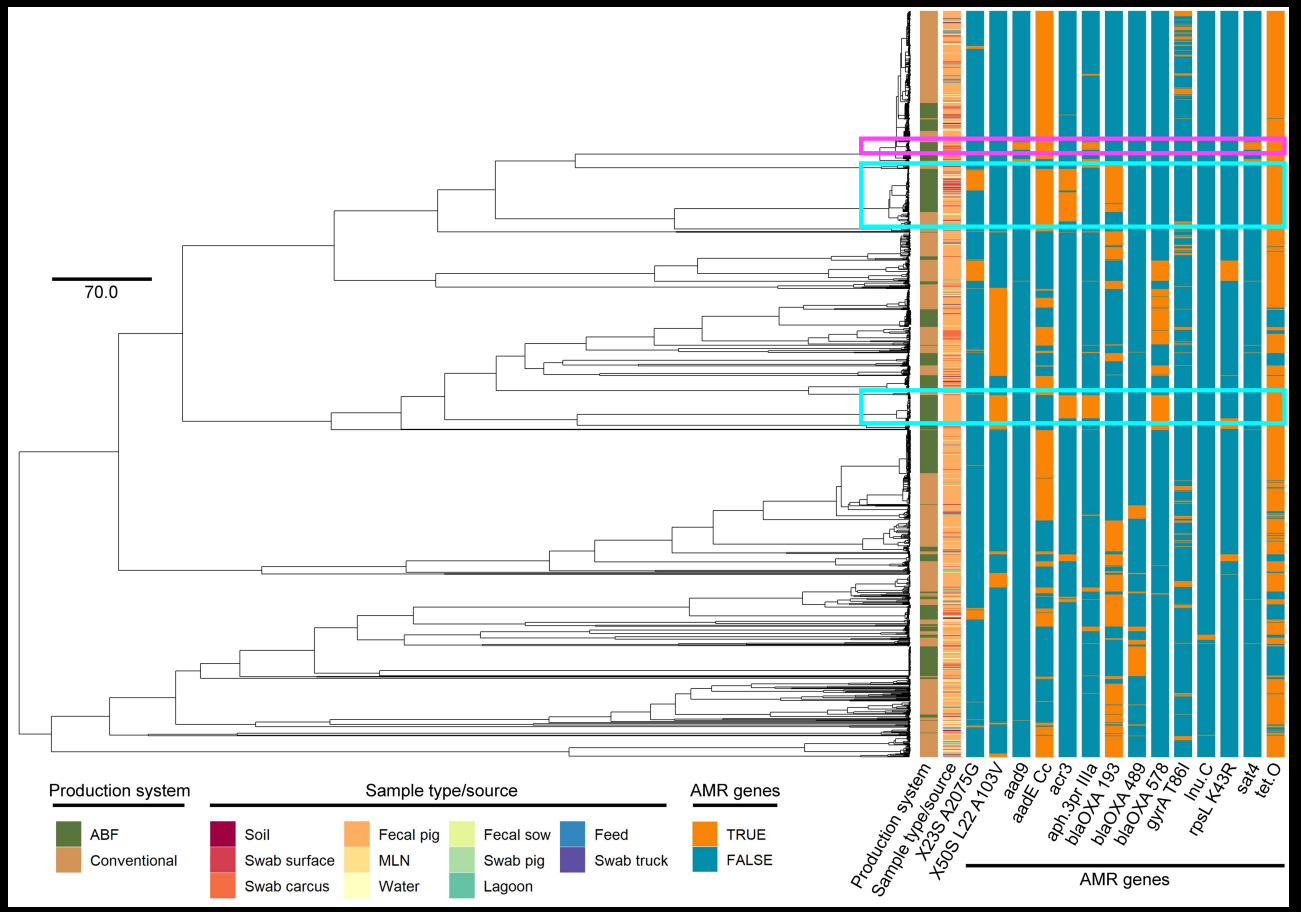
Model with all main effects plus two interaction terms:

- *gyrA* T86I X ABF
- *23S rRNA* A2075G X ABF

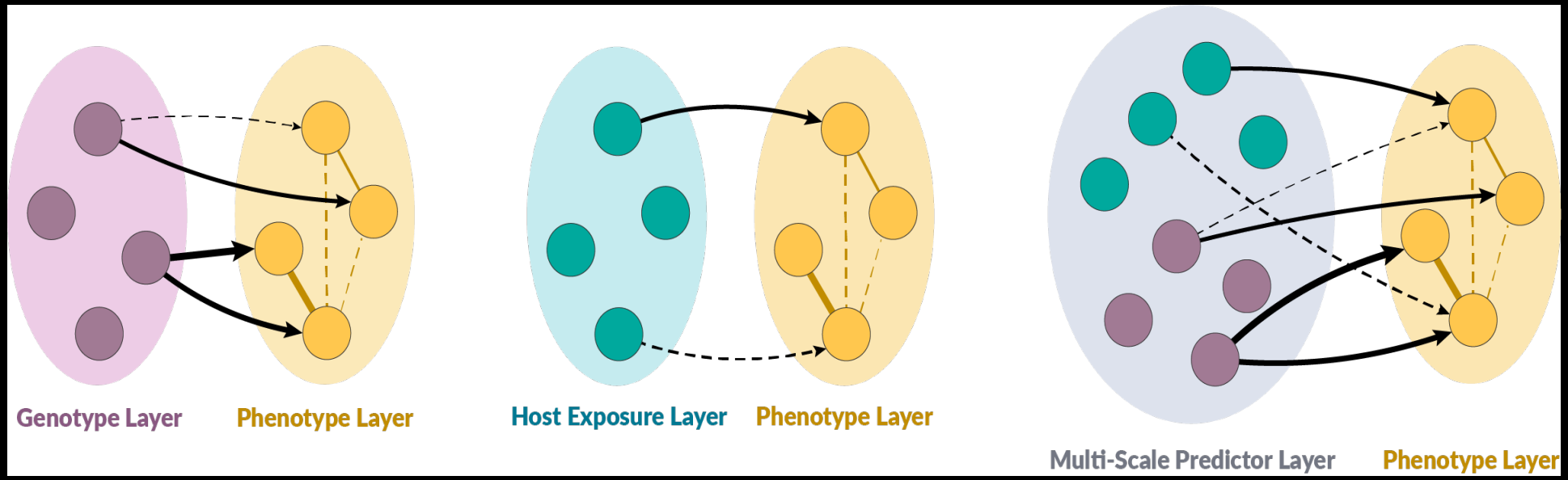


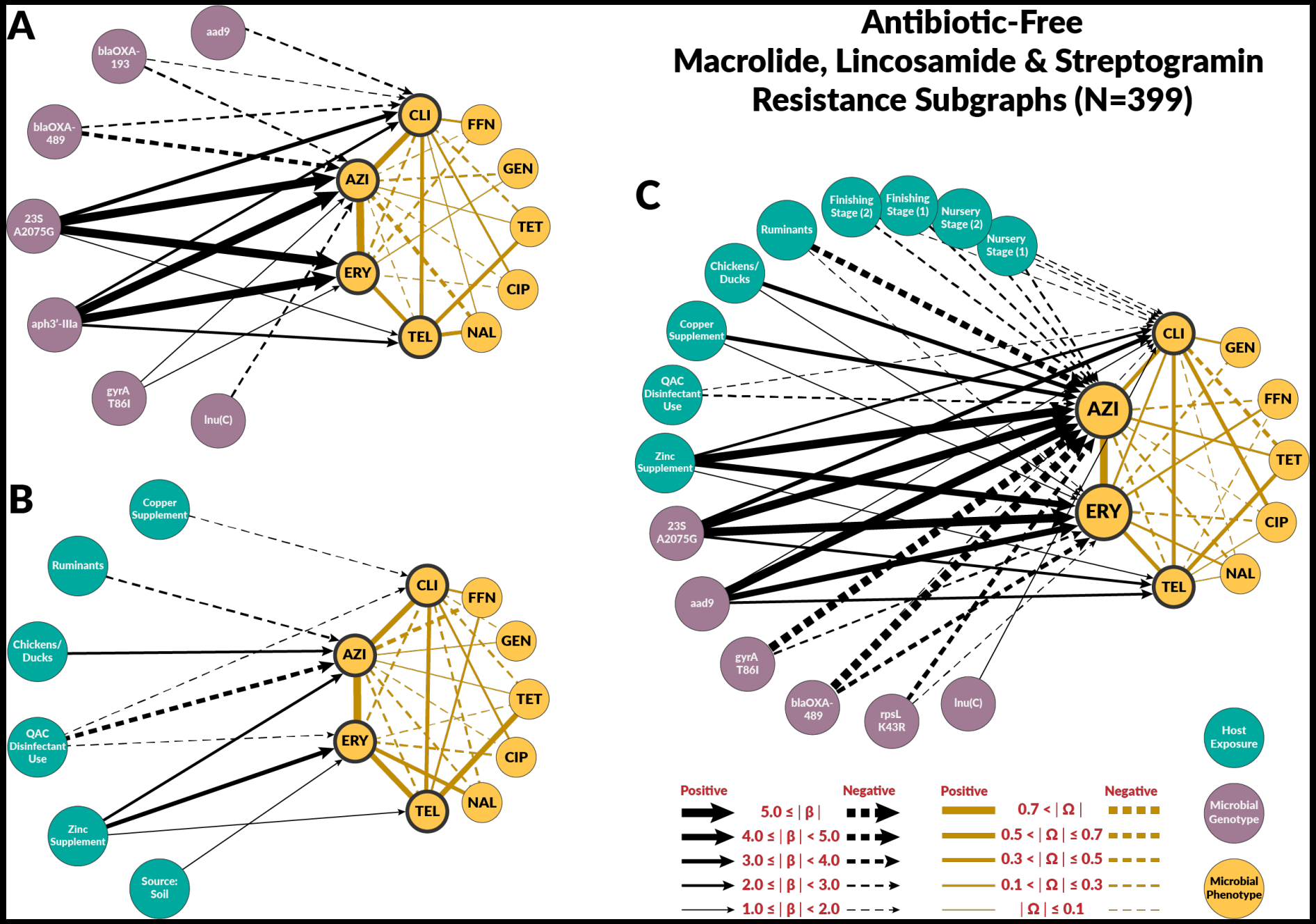
Non-Neutral Fitness Effects also Observed for *acr3*, *aad9* & *sat4*

Feature		MLE (95% CI)	Freq.
acr3	arsenic	1.033 (1.012, 1.050)	0.116
aad9	aminoglycoside	1.026 (1.012, 1.050)	0.016
sat4	aminoglycoside	0.992 (0.974, 0.993)	0.019



Co-selection? Multi-Layered Gaussian Chain Graph Framework for AMR Epidemiology




A
aad9
blaOXA-193
blaOXA-489
23S A2075G
aph3'-IIIa
gyrA T86I
lnu(C)
CLI
AZI
ERY
TEL
FFN
GEN
TET
CIP
NAL
B
Copper Supplement
Ruminants
Chickens/ Ducks
QAC Disinfectant Use
Zinc Supplement
Source: Soil
CLI
AZI
ERY
TEL
FFN
GEN
TET
CIP
NAL
Antibiotic-Free
Macrolide, Lincosamide & Streptogramin
Resistance Subgraphs (N=399)
C
Finishing Stage (2)
Finishing Stage (1)
Nursery Stage (2)
Nursery Stage (1)
Ruminants
Chickens/ Ducks
Copper Supplement
QAC Disinfectant Use
Zinc Supplement
23S A2075G
aad9
gyrA T86I
blaOXA-489
rpsL K43R
lnu(C)
CLI
AZI
ERY
TEL
GEN
FFN
TET
CIP
NAL
Positive
Negative
5.0 ≤ | β |
4.0 ≤ | β | < 5.0
3.0 ≤ | β | < 4.0
2.0 ≤ | β | < 3.0
1.0 ≤ | β | < 2.0
Positive
Negative
0.7 < | Ω |
0.5 < | Ω | ≤ 0.7
0.3 < | Ω | ≤ 0.5
0.1 < | Ω | ≤ 0.3
| Ω | ≤ 0.1
Host Exposure
Microbial Genotype
Microbial Phenotype

- G x E = P
  - Fitness advantage for *gyrA* T86I mostly attributable to beneficial effect among *C. coli* from conventional farms
  - Slight ***advantageous*** fitness effect for *23S rRNA* A2075G in *C. coli* isolates from ABF farms
- Some resistant features have neutral fitness effect
- Bottom-up approach to infer effectiveness of antibiotic stewardship interventions?

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FDA

NIH National Institutes of Health

CDC Centers for Disease Control and Prevention

CMI

TriCEM Triangle Center for Evolutionary Medicine